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24 April 2015

Version of attached file:

Accepted Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Daunton, R. and Gallant, A. J. and Katakya, R. and Wood, D. (2012) 'A multifunctional microgripper capable of simultaneous single cell manipulation and associated ion sensing.', MRS proceedings., 1463 .
mrss12-1463-qq03-03.

Further information on publisher's website:

<http://dx.doi.org/10.1557/opl.2012.1306>

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A Multifunctional Microgripper Capable of Simultaneous Single Cell Manipulation and Associated Ion Sensing

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ABSTRACT

The successful modification of the tips of a cellular microgripper into ion selective electrodes capable of sensing calcium ions at concentrations as low as 8×10^{-5} M is described. The modification involves applying the process of adding the components of all solid state ion selective electrodes. Specifically, poly(3,4-ethylenedioxythiophene) (PEDOT) is added to a gold electrode protruding from the microgripper tip; this is then coated with a poly(vinyl chloride) PVC based calcium selective membrane. Excellent Nernstian response was observed from our devices, with calibration slopes of 29.5 ± 2.5 mV/dec.

INTRODUCTION

Detecting the changes in intra- and extra-cellular ion concentration associated with cell signaling has always been of great interest, with many different methods being utilized. Potentiometric sensors are particularly advantageous in this area due to their comparatively small size, portability, low energy consumption and low cost [1]. Ion selective electrodes (ISEs) fall into this category.

Conventional ISEs contain an internal filling solution between the electrical contact and the selective membrane [2]. While these show good detection limits they are difficult to miniaturize and often have durability issues due to leaking of the internal solution, as well as issues associated with the aging of the internal electrolyte. This led to the development of all solid-state ion selective electrodes (ASSISEs). Due to the potential instability occurring when the selective membrane was directly applied to the electrical contact, a transducer material was added. This ion-to-electron transduction process occurs asymmetrically (Figure 1), so an intermediate layer with sufficiently high redox capacitance is required to minimize the polarizability of the solid contact. This led to the inclusion of conducting polymers (CPs) to the ASSISE design.

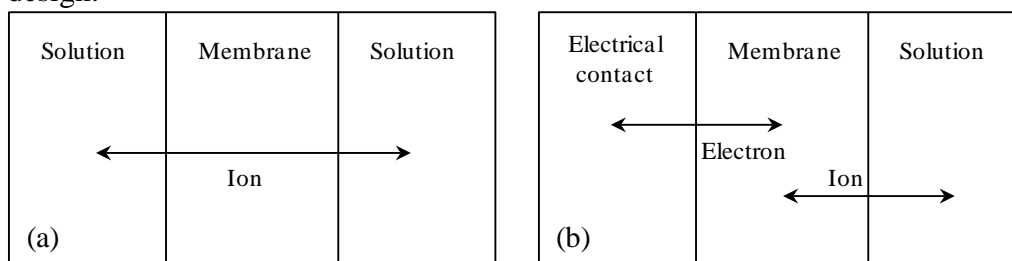


Figure 1 – Schematic showing the (a) symmetrical ion movement in a conventional ISE and (b) the asymmetrical ion-to-electron movement in an ASSISE.

While there are many types of ASSISEs, the most popular rely on glassy carbon, gold or platinum as the electrical contact; poly(3,4-ethylenedioxythiophene) (PEDOT) as the CP and poly(vinyl chloride) (PVC) as the membrane base [1]. The choice in the components within the

ion selective membrane (ISM) is complex and will not be discussed here; however a plasticizer, free ionophore and ionic site must be included within the membrane base [3]. The plasticizer is needed to set the membrane; the ionophore is the sensing element and determines the ISE selectivity, (it can be either charged or neutral); and the ionic site is a hydrophobic ion with the opposite charge to that of the measured ion, and is required to ensure charge neutrality.

Previously published work has demonstrated the successful operation of a microgripper device in manipulating micron scale objects [4]. Also published is the successful incorporation of an electrode protruding at the microgripper tip which can be used as a working electrode in a 3-electrode electrochemical set up [5]. This paper focuses on modifying the electrode at the microgripper tip into an ISE that has the ability to sense calcium ions.

EXPERIMENTAL

The fabrication of the microgripper was completed via a process described previously [4] but with an additional electroplating step, after the metallization but before the patterning, to define the electrode. Figure 2 shows the position of the electrode with respect to the microgripper tip. This gold electrode is then modified into an ISE via the route discussed below.

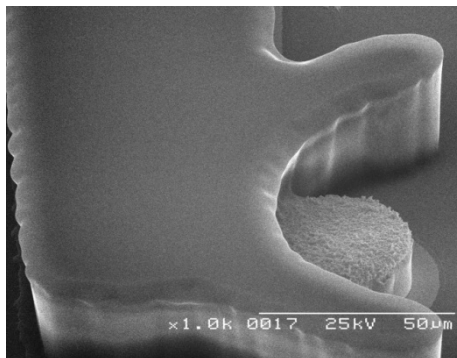


Figure 2 – SEM image of the microgripper tip with a protruding gold electrode.

Cyclic voltammograms (CVs) were obtained using a multichannel potentiostat (Perkin Elmer Model 283). The potentiometric measurements were performed using a digital multimeter (Thurly Thandar Instruments Model 1705) connected to a computer for data acquisition. A solid silver-silver chloride electrode was used as the reference electrode (RE) and a platinum flag electrode was used as the counter electrode. The bare gold electrode fabricated into the microgripper design was used as the working electrode (WE) in the electrochemical setup. All measurements were carried out in a 3-electrode cell arrangement with the RE and WE mounted onto a printed circuit board to maintain a constant distance between them. All chemicals were of analytical grade and were used without further purification.

The gold microgripper tip was modified into a Ca^{2+} ISE via the following route. The CP used in this study was PEDOT doped with sodium poly(styrene sulfonate) (NaPSS). This was electropolymerically deposited onto the gold WE. 0.01 M EDOT + 5 mg/ML NaPSS (aq) (mixed by stirring for 3 hours) was de-aerated with argon for 15 min. A 3 electrode set up was used to apply a cycling potential between -0.7 – 1.0 V at a scan rate of 50 mVs^{-1} for 5 cycles. The CP layer was allowed to dry (1 hour) and then the ISM was deposited dropwise using a 10 μL Hamilton syringe. The composition of the ISM is shown in Table I. The ISM was dried overnight (12 hours) and then conditioned in an open circuit in 0.1 M solution of CaCl_2 for 12 hours before calibrating. Calibrations were done by 10 fold dilutions of 0.1 M CaCl_2 + 0.1 M KCl (aq) with 0.1 M KCl (aq) solution.

Table I – ISM components made up to a total of 200 mg in 3 ml of tetrahydrofuran (THF).

ISM component	Chemical	Quantity / % wt
Base membrane	Poly (vinyl chloride) (PVC)	32.6
Plasticizer	o-Nitrophenyloctylether (o-NPOE)	65.6
Ionophore	ETH 1001 (Calcium ionophore I)	1.3
Ionic Site	Potassium tetrakis(4-chlorophenyl)borate (KTPClPB)	0.5

DISCUSSION

Electropolymerization of PEDOT

The experimental parameters of the electropolymerization of PEDOT greatly affect the surface morphology and detailed studies have been undertaken to assess these [6]. While aprotic solvents tend to yield smooth surfaces, aqueous solutions were used in this case to ensure good compatibility with biological media. Figure 3 shows the CV of the electropolymerization of PEDOT doped with NaPSS.

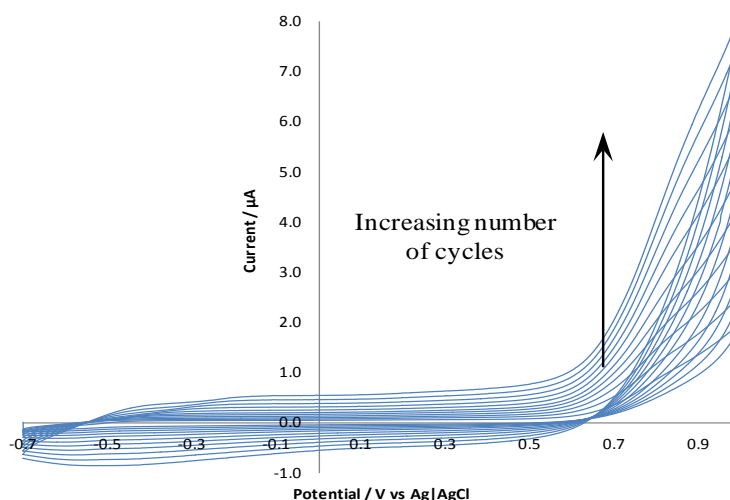


Figure 3 – Electropolymerization of 0.01 M EDOT + 5mg/mL NaPSS (aq) on the microgripper device

In the first cycle there is a peak at 0.6 V which is the oxidation of the EDOT monomer. At this point EDOT loses an electron and forms a reactive radical intermediate. This then reacts immediately with an available EDOT monomer to form a dimer species as shown in Figure 4. As the potential is swept to -0.7 V the dimer structure is reduced to give the charge stable form. As the cycles progress, more and more EDOT monomers are added to the polymer chain resulting in the formation of PEDOT. As the number of cycles increases an additional peak is seen around -0.4 V which is the oxidation of PEDOT (a redox active analyte in its own right). As the PEDOT layer gets thicker, (and hence the PEDOT concentration increases), the peak current increases.

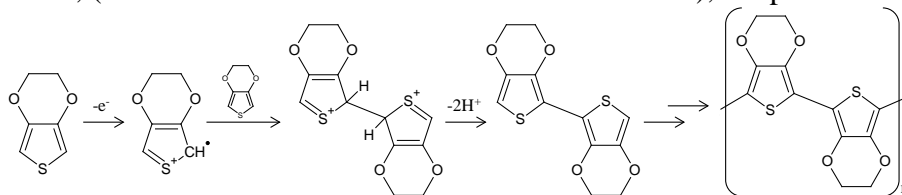


Figure 4 – Reaction scheme of the polymerization of EDOT to PEDOT

Calibration of Ca^{2+} microgripper-ISEs

For historical reasons the measured potential is typically referred to as electromotive force (EMF). At 25 °C the EMF, as predicted by the Nernst equation (1), will increase by 59.1 mV/ z_i for every 10 fold increase in the activity (a_i) of the ion of interest (i); where z_i is the charge of that ion, T is the temperature (K), R is the universal gas constant, F is Faraday's constant and E^0 is the standard cell potential and is a constant.

$$EMF = E^0 + \frac{RT}{z_i F} \ln a_i = E^0 + \frac{2.303RT}{z_i F} \log a_i = E^0 + \frac{0.0591}{z_i} \log a_i \quad (1)$$

The total potential of the ISE is determined as the sum of all phase boundary potentials:

$$EMF = \sum E_{PB} \quad (2)$$

$$\approx E_{\text{const}} + E_{PB(\text{ISE membrane/sample})}$$

As only one phase boundary occurs between the sample and a neighboring phase all other phase boundaries will add up to a constant, E_{const} .

Figure 5 shows the first and repeat calibration curves of a Ca^{2+} microgripper-ISE. Here it can be seen that the slope of the original calibration is 29 mV/dec, which is very close to the 29.5 mV/dec predicted by the Nernst equation for a divalent ion. The limit of detection (determined at the point of intersection between the two linear areas on the plot) is calculated as 0.14 mM with a response time of 98 s.

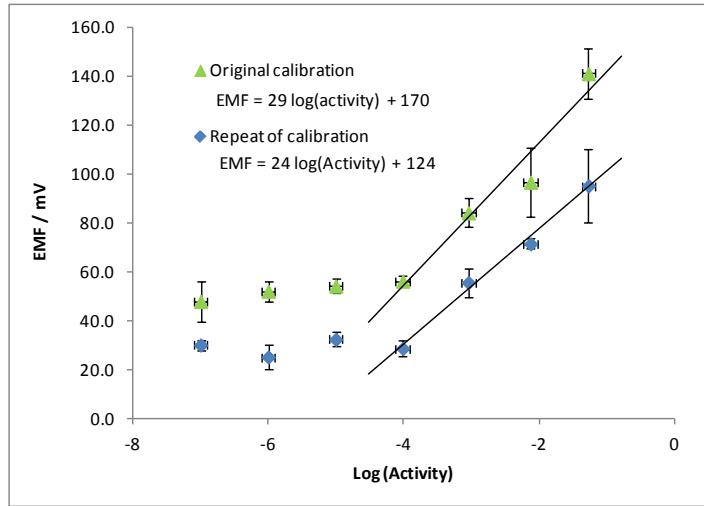


Figure 5 – Calibration of Ca^{2+} microgripper-ISE

When the device is recalibrated to check reproducibility within a device it was found that the slope changed to 24 mV/dec, which is still within accepted Nernstian behavior. The limit of detection was calculated as 0.12 mM with a response time of 78 s. All of these details are within standard limitations for ISEs; however the absolute values of EMF for a specific ion activity display significant potential drift. For example, at 10^{-1} M the EMF in the original calibration was 141 mV; this decreased to 95 mV when repeated. This highlights a problem with using this device to detect calcium concentrations in samples of unknown concentration as this potential drift is not consistent between each calibration. The cause for this drift is in the stability of the ISM|solution potential boundary.

Comparison of ISM deposition and calibration response

SEM images of several ISE-modified electrode tips that had varying calibration responses were taken to determine the trend. It was observed that thicker ISMs often gave poorer detection limits (as shown in Figure 6). This is likely due to the limitations of diffusion of the ion through the ISM reducing the sensitivity of the ISM|solution potential. The irreproducible super-Nernstian response of 59mV/dec is significantly greater than that predicted, an effect seen by other groups with thick ISMs [7]. Interestingly the response times for this device were quicker than for those with thinner ISMs. This can be attributed to the smoothness of the surface. In general the smoother the ISMs are; the more stable and faster the potentiometric response is [8]. It is also important to note from Figure 6 that the microgripper arms are fused together by the ISM rendering them useless as manipulators.

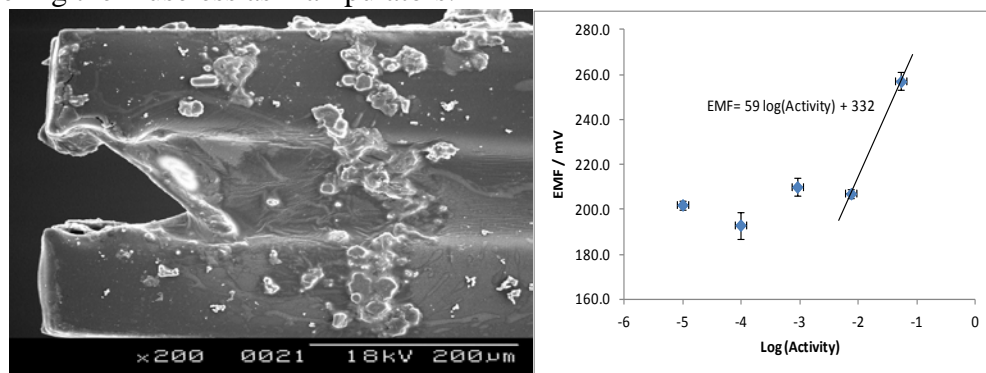


Figure 6 – SEM image of microgripper-ISE device with thick ISM deposition with corresponding calibration response.

Figure 7 shows the response of a much thinner ISM deposition. Here the device operates well, with a slope of 30 mV/dec, a detection limit of 8×10^{-5} M and response time of 93 s. Comparing the slope response in Figure 7 with that from the original calibration in Figure 5 it can be seen that devices with similar ISM thicknesses give similar slope responses. In fact all devices with thin ISMs gave responses of 29.5 ± 2.5 mV/dec.

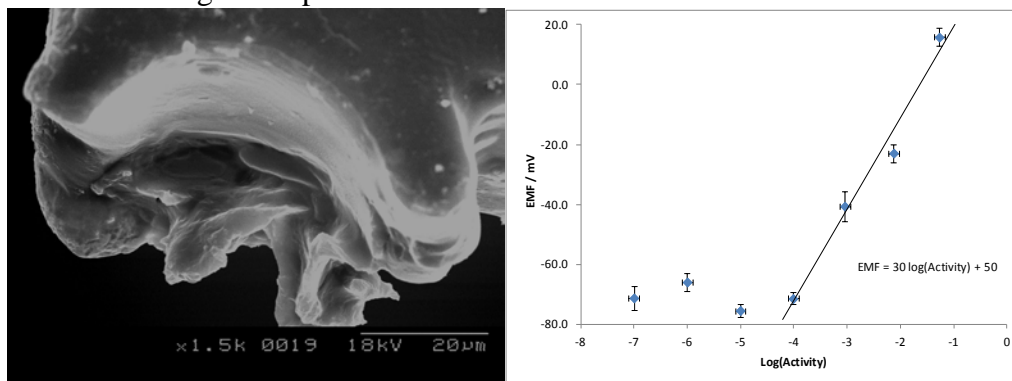


Figure 7 – SEM image of microgripper-ISE device with thin ISM deposition with corresponding calibration response.

The ability to control the ISM deposition is very user dependent and generally 50% of all devices fabricated to ISE stage fail to give a response at all. This again can be attributed to the thickness of the ISM. Figure 8 shows the two extremes of ISM deposition. Figure 8(a) shows a very thick ISM deposition where the electrode is buried so deep within the ISM that the ISE is unable to detect changes in analyte activity at all. At the other extreme, Figure 8(b) shows a very thin ISM deposition. This gave an erratic calibration response and, on inspection, the SEM image

indicated that the ISM had been delaminated from the electrode surface, possibly removing the PEDOT, leaving behind a very unstable ISE.

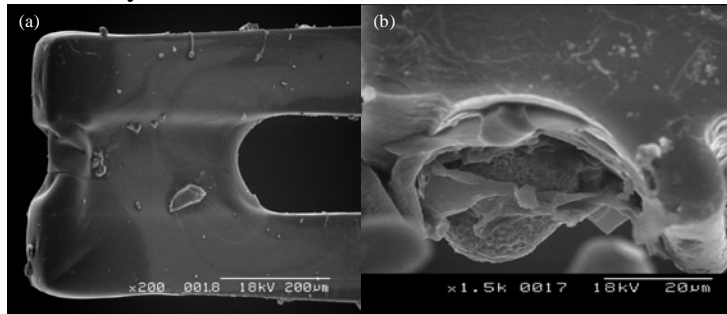


Figure 8 – SEM images of (a) very thick ISM deposition and (b) very thin ISM deposition which delaminated during conditioning.

CONCLUSIONS

The tip of the microgripper device has been successfully modified to behave as an ISE. PEDOT was used as the CP to aid the ion-to-electron signal transduction. The ISM was based on PVC and contained an ionophore to give good selectivity towards calcium ions. The calibration response of these Ca^{2+} microgripper-ISEs was within that predicted by the Nernst equation (29.5 ± 2.5 mV/dec); and gave good detection limits (8×10^{-5} M) and response times (93 s). However, the ISM deposition stage of the fabrication was very user dependent and too thick ISMs significantly reduced the detection limits and the slope response became super-Nernstian. Another limitation of these devices is that between uses significant potential drift was observed, meaning that in their present state determining the activity of samples with unknown concentrations would be inaccurate.

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